Clinical and Positron Emission Tomography of Parkinson's Disease Caused by *LRRK2*

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We have recently identified mutations in a gene leucine-rich repeat kinase–2 (*LRRK2*), which cause autosomal dominant Parkinson's disease. Here, we describe two families with autosomal dominant Parkinson's disease caused by a *LRRK2* G2019S mutation. We present here a clinical description of patients, including 6-^{18}F-fluoro-L-dopa positron emission tomography and discuss the potential implications of this mutation, which alters a conserved residue in a domain required for kinase activation.

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Parkinson's disease (PD) is a severe progressive neurodegenerative disorder characterized by bradykinesia, postural instability, tremor, and rigidity. The histological features of PD are loss of dopaminergic neurons from the substantia nigra and the presence of brainstem neuronal Lewy body inclusions.

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PD is largely a sporadic disease; however, the identification of familial forms of parkinsonism and detection of causative gene mutations has proved a valuable step toward understanding the molecular processes underlying PD.

We have recently identified mutations within *LRRK2* (leucine-rich repeat kinase–2), which cause a disease resembling typical PD, both clinically and neuropathologically.[1,2] Subsequent to this discovery Zimprich and colleagues also published mutations in *LRRK2* causing disease in five, possibly six, families.[3]

Because mutations in *LRRK2* are a newly identified cause of PD, the description of novel mutations and associated clinical features is informative. We describe here the identification of a novel *LRRK2* mutation (G2019S) in two apparently unrelated families with PD and present a clinical description of family members.

Materials and Methods

Subjects

These studies were performed under an approved protocol, and informed consent was obtained from each subject. Standard neurological clinical examination of subjects was performed by three of the authors (G.L., R.L.N., and G.A.) for Patients 292-18, 292-22, 292-23, 292-24, 292-25, and 415-15. The diagnosis of PD was based on published criteria.[4]

Positron Emission Tomography Scanning

To measure 6-fluorodopa (6-FD) uptake, subjects were pretreated with 200mg of carbidopa, and 14 ± 3.5 (mean ± standard deviation) mCi of 6-FD was infused over 90 seconds. Images were acquired from 1.5 minutes after the start of infusion up to 90 minutes later (25 images total).

All images were attenuation-corrected and reconstructed (32 planes, 6.5mm full-width at half-maximum). Further processing of 6-^{18}F-dopa data used SPM99 software (Wellcome Department of Cognitive Neurology). Scans were aligned and registered using a rigid affine transformation, coregistered, and affine-normalized to an in-house template. The kinetic rate constant K_i for dopaminergic uptake was calculated voxel-by-voxel using a linear fit based on the Patlak method using the time activity curve in an occipital reference region as the input function as described previously.[5] Average values in regions of interest defining left and right caudate and putamen in normalized space were extracted and adjusted for age and sex.

Molecular Genetic Analysis

DNA was isolated from whole blood, and polymerase chain reaction (PCR) amplification of exons was performed as previously described.[2] Cycle sequencing in forward and reverse directions was performed on purified PCR products and run on an ABI3100 sequencer (Applied Biosystems, Foster City, CA). Assay of the G2019S mutation in members of Families 292 and 415 and in 777 normal control subjects was performed by PCR amplification with primer pair DKFZp434 40F 5′-TTTTGATGCTTGACATAGTGGAC-3′ and DK-

FZp434 40R 5′-CACATCTGAGGTCAGTGGTTATC-3′ followed by restriction digest with *Sfc*I. Wild-type products produced fragments of 228bp and 109bp and the mutant produced fragments of 207bp, 109bp and 21bp, discernable on a 4% agarose gel.

Sixteen single-nucleotide polymorphisms (SNPs) flanking G2019S were chosen spanning 1.65Mb. Fourteen of these SNPs were selected from dbSNP (http://www.ncbi.nlm.nih.gov/SNP/) based on location, informativity, and validation. SNPs LYS543ARG and LNGp1 were identified during the course of a positional cloning project.[2] Each SNP was typed by direct sequencing (primers and conditions available upon request). Where available, DNA from spouses, siblings, and offspring of patients was also genotyped to establish phase (data not shown).

Results

Clinical Examination

Clinical characteristics of affected family members are shown in Table 1.

FAMILY 292. This North American family has ancestors on both sides who migrated from England. Disease mode of inheritance is consistent with autosomal dominant and consanguinity is denied. The mother of affected individuals involved in this study died at the age of 76 years, long before this research began. Family members report that she had symptoms about 20 years before her death and that she had severe tremor, shuffling, and postural instability.

FAMILY 415. The mode of inheritance in this family is consistent with autosomal dominant disease and consanguinity is denied. The family is of Ashkenazi Jewish ancestry and they migrated from Russia. Only affected Subject 415-15 was available for examination. The age of onset in unsampled affected family members ranged from mid-fifties to late seventies (Fig). All were reported to have had a tremor dominant clinical presentation, initially unilateral progressing to include both sides of the body. Subject 415-15 showed good initial response to L-Dopa but 15 years after the onset of symptoms had significant drug-related fluctuations and problems when walking during the "off" period because of poor balance, freezing, and drug-induced dyskinesias. As medical management was not offering a satisfactory quality of life, she opted for a stereotactic pallidotomy. After surgery, there was a reduction in drug-induced dyskinesias on the left side and a reduction in fluctuations. In the 10 years after her surgery, her balance deteriorated further, and after several falls she began using a wheelchair, at the age of 76 years.

Positron Emission Tomography Imaging

Imaging of presynaptic dopamine synthesis showed reductions in Subjects 292-22 and 292-23, in both cases with maximum reduction in the putamen, showing a caudate-putamen gradient similar to that in idiopathic parkinsonism (Table 2). Uptake reduction was more extensive on the left for Subject 292-22. Percentage reduction relative to the age-adjusted means from a group of 56 normal controls (age range, 20–80 years) is shown (see Table 1). Scans of a genetically unaffected sibling and of three next-generation family members did not show abnormalities in dopamine synthesis.

Molecular Genetic Analysis

Sequencing of the coding region of *LRRK2* showed a missense mutation G2019S (based on AY792511) present in affected family members 292-18, 292-22, 292-23, 292-25, and 415-15 but not affected family member 292-24 (see Fig). Restriction digest assay of 1,554 chromosomes failed to show the presence of this

Table 1. Clinical Characteristics of Affected Family Members

Subject No.	Age at Onset (yr)	Age at Last Examination (yr)	Presenting Tremor	Rigidity	L-Dopa Response (age at treatment, yr)	Drug-Induced Dyskinesia[a]	Bradykinesia	Motor UPDRS	Hoehn and Yahr Staging	UPSIT
292-18	73	78	RUE	Moderate	Not tried	Not treated	Moderate	22/56	2	32/40
292-22	63	72	LLE	Mild	Beneficial (63)	0	Mild	13/56	1.5	32/40
292-23	45	70	LLE	Moderate	Beneficial (52)	3	Mild	21/56	2	6/40
292-24	58	68	RLE	Marked	Beneficial (68)	0	Moderate	29/56	2	13/40
292-25	53	68	RUE	Marked	Beneficial (60)	0	Moderate	22/56	2	—
415-15	54	76	LLE	Marked	Beneficial (69)	4	Marked	33/56	5	26/40

All characteristics are at last examination with the exception of presenting tremor, which is the location of tremor at initial presentation. The initial symptom in all patients was tremor.

[a]Drug-induced dyskinesia-rating taken from question 32 of the UPDRS, proportion of the waking day with dyskinesias; 0 none; 1, 1–25%; 2, 26–50%; 3, 51–75%; 4, 76–100%. Motor UPDRS is in the "on" state.

UPDRS = Unified Parkinson's Disease Rating Scale; UPSIT = University of Pennsylvania Smell Identification Test; RUE = right upper extremity; RLE = right lower extremity; LUE = left upper extremity; LLE = left lower extremity.

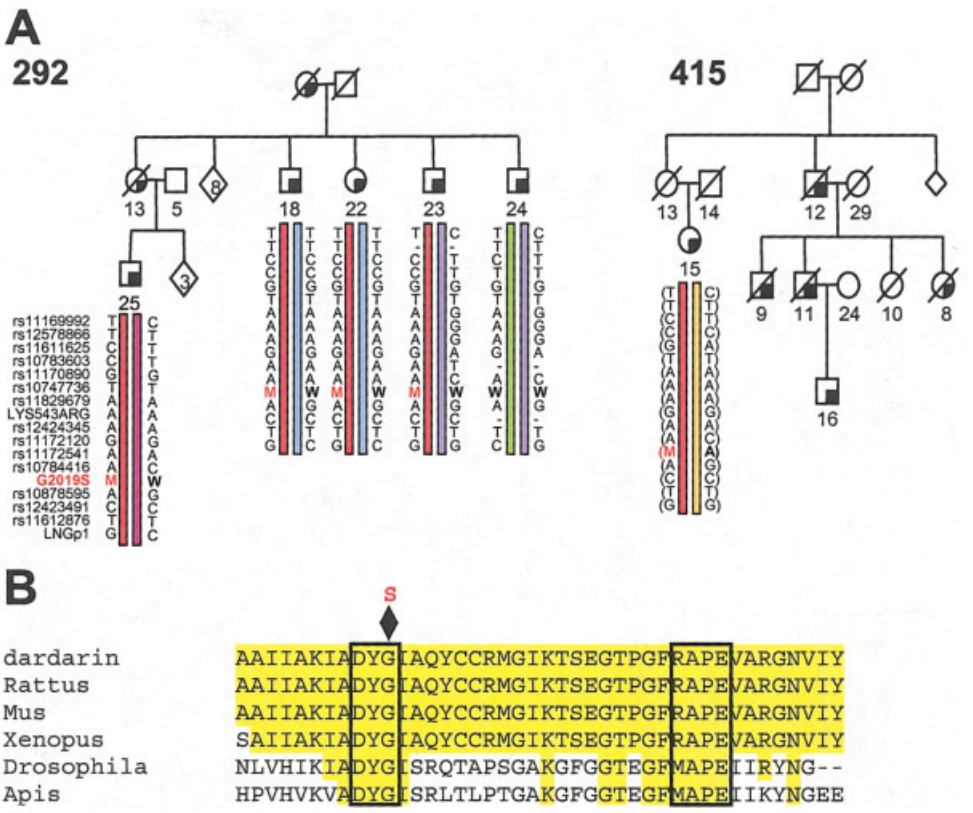

Fig. (A) Abridged pedigrees for Families 292 and 415 showing single-nucleotide polymorphism haplotypes around the G2019S mutation. Phase-known haplotypes were determined by genotyping siblings, spouses, and children. Phase is unknown (indicated by parentheses) in Subject 415-15, but genotypes are consistent although not indicative of a common interkindred disease haplotype. Family member 292-24 is affected with typical Parkinson's disease but does not carry the G2019S mutation. (B) Protein alignment of dardarin and orthologs. Boxed region represents the highly conserved domain of the kinase activation loop; the highlighted region shows high degree of homology. The Gly2019Ser mutation is indicated in red.

variant in controls but confirmed the presence of G2019S in all affected family members previously shown to carry the mutation by sequence analysis. All remaining 50 exons within *LRRK2* were mutation free.

Genotyping of 16 SNPs flanking the G2019S mutation was consistent with a common disease haplotype shared between mutation carriers in the two families (see Fig).

Discussion

We describe here the identification of a missense mutation G2019S in *LRRK2* encoding dardarin as a cause of PD in two families with autosomal dominant disease. We provide data consistent but not indicative of a common founder shared by these families. A single affected member of Family 415 carries the G2019S mutation, as do four of five affected family members in kindred 292. The failure to identify this mutation in the fifth affected member, 292-24, suggests that either this mutation is a benign variant or it is pathogenic and disease in member 292-24 represents a phenocopy. Three pieces of data support this variant as pathogenic. First, 2019S is not present in 1,554 control chromosomes; second, 2019S has been identified in two apparently unrelated families with autosomal dominant PD; and third, 2019S alters an amino acid that is a highly conserved, key residue involved in the activation of kinases. Although phenocopy in families with genetic disease is unexpected, it is not unprecedented. Notably, disease phenocopy was present in the original α-synuclein A53T kindred[6] and in a large family with presenile dementia caused by APP mutation.[7] Our genetic data are consistent with the hypotheses that this mutation arose from a single founder, but we cannot rule out the possibility that in both instances the mutation has arisen independently on a frequently occurring haplotype.

This residue, the third in the D-Y-G motif of the activation loop of this kinase domain, is highly conserved across all orthologs of dardarin and all eukaryotic kinases.[8] Although the effects of this mutation on dardarin are unclear, it may result in constitutive activation or inactivation of kinase activity or alter substrate specificity. Regardless of effect, this mutation di-

Table 2. Percentage Reduction of Age-Adjusted F-dopa Uptake in Striatal Regions of Interest Relative to 56 Normal Controls, age range 20 to 80 Years

Subject No.	L Caudate	R Caudate	L Putamen	R Putamen
292-22	−10.0	−8.0	−46.7	−26.0
292-23	−28.6	−39.5	−64.2	−69.9

rectly implicates the kinase activity of dardarin in pathogenicity. As such, this mutation may offer molecular insight by facilitating functional modeling in silico, in vitro, and in vivo and provide a tool with which to identify the substrates of this kinase.

The phenotype of 2019S carriers closely resembles that of typical PD and previously described cases with LRRK2 mutations.[1-3] The mean age at onset observed within 2019S-positive cases is 58, similar to that described previously for dardarin linked cases.[1]; however, the earliest case, presenting at 45 years of age, is the youngest mutation-positive case identified to date. Consistent with previous *LRRK2*-linked patients, the presenting symptom is tremor, often of the foot or leg and the disease progresses slowly. Approximately 10 years after diagnosis patients (292-22, 292-23, 292-25, and 415-15) reported problems with balance. It appears that L-dopa treatment was initially successful; however, two patients treated for more than 7 years suffer from drug-induced dyskinesias for more than 50% of the waking day. Positron emission tomography showed uptake reductions in caudate and putamen somewhat less than those seen in typical PD of comparable duration[9]; this may reflect the relatively slow progression noted in the patients examined here. Aside from the motoric component of this presentation, University of Pennsylvania Smell Identification Tests show varying degrees of microsmia and anosmia.

In summary, we present data showing mutation of *LRRK2*, encoding dardarin, as a cause of PD in two families from North America. This mutation, which alters a key residue involved in kinase activation, implicates the kinase activity of this protein in the pathogenic process for all PD-causing *LRRK2* mutations.

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